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EVALUATION OF THE PRESERVATION POTENTIAL OF POTASH (*KANWA*) ON SHELF LIFE OF PASTEURISED MILK INOCULATED WITH 2% *LACTOBACILLUS PLANTARUM AND LACTOBACILLUS FERMENTUM*

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ABSTRACT

The study aimed at evaluating the potency of potash (kanwa) on shelf life of pasteurised milk. Following inoculation of milk with 2% L. fermentum and L. plantarum, the milk was divided into seven portions designated as A-G each containing 50ml of the sample. They were treated as follows; A= untreated, B= 0.2% v/v potash, C=0.4% v/v pot ash, D=0.2%v/v sodium benzoate, E= untreated refrigerated, F= 0.2% v/v potash G=0.2% v/v sodium benzoate. Samples A-D were stored at ambient temperature (mean=35°C) while E-G were stored at refrigerated temperature (10°C). All the samples were analyzed for physicochemical, bacteriological and organoleptic properties at 24hours interval for 5days.

Introduction:

Milk has been defined as the lacteal secretion practically free from colostrums obtained by the complete milking of one or more healthy cows (USDA, 2005). Milk and milk products provide significant amount of protein and most micronutrients, including calcium, B. group vitamin (particularly riboflavin and B12 but also thiamin, niacin, B6), vitamin A, iodine, Magnesium, phosphorus, potassium and zinc. (Anita, 2001).

Milk is a highly nutritious food which make it an ideal medium for microbial growth. Thus, it often deteriorates and

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The results revealed that the mean pH ranged from 7.21±0.01 - 4.41±0.01. Highest decrease (p>0.05) in pH (4.41±0.01) was observed in sample A while lowest decrease (p>0.05) was observed in F (6.89±0.01). The titratable acidity (T.A) ranged from 0.22±0.02-1.19±0.06%, highest increased in T.A (1.19±0.06%) was recorded in A while lowest increased (0.23±0.01%) was observed in F. The viscosity ranged from 57±5.77 - 10±0.00Cp, highest increase (57±5.77Cp) was observed in A while lowest increase (14.0±0.00) was recorded in E, D and F at 24hrs of storage. The LAB count was 5.08 log cfu/ml at 0 hour. LAB count reduction was observed in all samples except A. Highest (0.82 log cfu/ml) and lowest (0.01 log cfu/ml) reduction were recorded in samples G and B respectively at 4 days of storage. The results of organoleptic scores showed significant (p<0.05) decrease in the overall acceptability in all samples as the storage progress. Highest score (8.7±0.9) was recorded in G while sample A showed lowest score (5.3±1.7) at 5days. Refrigeration at 10°C in combination with additives (0.2% sodium benzoate and 0.2% kanwa) were shown to be the most effective means of preserving pasteurised milk.

Keywords: *Potash, Pasteurised milk, Shelf life, L. plantarum L. fermentum, Preservation*

becomes unsuitable for processing and human consumption (Food and Agriculture Organization, 2001). Milk and milk products can harbor a variety of microorganisms and can be important sources of food-borne pathogens (Oliver *et al.,* 2005). Researchers have successfully deduced methods to preserve milk with biological and chemical methods to improve the shelf-life of milk. The use of 0.8% proponic acid and 0.8% sodium benzoate in the preservation of cheese, and milk for 8 days have been reported by Joseph and Akinyosoye (1997). The scientific communities have given more attention towards the potential antimicrobial activities of natural products as a results of harmful effects of chemical preservatives to consumers because of the tendency to induce

allergic contact dermatitis, asthma and convulsion. (Fang *et al.,* 2008; Anand and Sati , 2013).

Kanwa is also known as Trona or sodium sesquicarbonate (Minka, 1999), is a naturally occurring alkaline rock salt. It is not only used as tenderizer but also as flavoring agent, food preservatives and prophylactic (Uzogara *et al.,* 1988). It has become a multipurpose as a major food supplement in most rural and urban home areas including food industries in Nigeria, as well as medicine (Omajali*et al.,* 2010).

The use of potash (*kanwa)* in preserving milk and milk products has not previously reported despite the fact that some rural habitant and milk hawkers used it as milk presevatives. There is limited published document as regards to its effects on physicochemical, bacteriological and organoleptic quality of milk. It is therefore imperative to investigate the effect of such additive on, shelf life and organoleptic quality of milk. The aim of this research was to evaluate the potency of potash (kanwa) on shelf life of pasteurised milk.

MATERIAL AND METHODS

Collection of samples

Fresh cow milk was collected in a sterile plastic container from Tudun Yola district Gwale Local Government Kano state. It was immediately placed in an ice box container and transported to microbiology laboratory, Bayero University Kano (BUK), for analysis.

The *Lactobacillus plantarum* used for this study was obtained from local yoghurt (*kindirmo*).

Sample processing

Pasteurisation and filtration of milk of fresh whole cow milk

The fresh milk in sterile plastic container was placed in water bath and heated at 62^0C for 30 minutes and allowed to cool down. The pasteurised milk was then filtered through sterilized membrane filter (sized 0.4µm) in order to remove the heat resistant bacteria as well as their spore that survived pasteurisation temperature.

Standardization of inoculum (*Lactobacillus plantarum)*

The isolated *Lactobacillus plantarum* was standardized by comparing the bacterial suspension with 0.5 Macfarland turbidity standard equivalent. This was carried out according to the method described by Cheese brough (2002) and Clinical Laboratory Standard Institute (CLSI) (2010).

Inoculation of pasteurised milk with 2%(v/v) *Lactobacillus plantarum*

The pasteurised filtered milk was then inoculated with 2% *Lactobacillus plantarum and lactobacillus fermentum* (0.5Macfarland standard). The inoculated milk was divided into seven portions designated as A- G each containing 50ml into sterilized glass bottles.

Treatment of inoculated milk with preservatives

The seven equal portions of inoculated milk were treated with different quantities of preservatives as follows; Sample A (untreated), sample B (0.2% potash solution (PS)), sample C with (0.4%)PS), sample D (0.2%) sodium benzoate (SB), sample E (untreated) , sample F (0.2% PS), and G (0.2% SB). Samples A-D were stored at room temperature (mean=35^{o}C) while samples E-G were refrigerated at 10^{0}C (mean temperature). All the samples were analyzed for Lactic acid bacteria (LAB) count, physicochemical analysis (pH, titratable acidity, viscosity and temperature) and sensory evaluations at 24h intervals for five days.

Determination of Physicochemical Parameters of treated and untreated pasteurised milk

All measurement of physicochemical parameters was carried out in triplicate determinations.

pH

The pH of pasteurised milk was determined at room temperature (32 $\pm$ 2^{0}C) using a digital pH meter (Jenway 3505). The pH meter was calibrated with buffer standards of pH 4.0 and pH 10.0 prior to use. Readings were recorded by immersing the probe in the sample until the reading stabilized.

Titratable acidity (T.A)

The titratable acidity was determined according to the method described by Association of Official Analytical Chemists (AOAC, 2005).

Viscosity

Viscometer (DV-E) was used to determine the viscosity of the milk samples. The spindle (size 4) was dropped in 100ml of pasteurised milk and the rotator was set for 100 resolutions per minutes. The rotator was allowed to swing for 15 seconds. The reading on the dials was taken 3 times for each sample. The average was calculated and then recorded.

Enumeration of lactic acid bacteria (LAB) of treated and untreated pasteurised milk

This was carried out according to the method described by Omafuvbe and Enyioha (2011) with slight modification in plating 10^{-3} dilution.

Evaluation of the Organoleptic Properties of treated and untreated pasteurised milk

Organoleptic evaluation of treated and untreated pasteurized milk were carried out using 9 point hedonic scales using the method described by David (2005). The appearance, taste, aroma, texture and general acceptability were scored by 5 panelists who were regular consumers of milk. The panelists were instructed to taste the pasteurised milk and express their views by scoring organoleptic attributes using the nine hedonic scales;9(like extremely), 8(like very much), 7(like moderately), 6(like slightly),5(neither like nor dislike), 4(dislike slightly) , 3(dislike moderately), 2(dislike very much), and 1(dislike extremely).

Statistical Analysis of Results

The results were analyzed using One way –Analysis of variance (ANOVA) at 5% probability level of significant using SPSS version 20. The mean separation was carried out using least significant difference (LSD).

Results and discussion

Table 1 presents the changes in physicochemical characteristics of pasteurised milk inoculated with 2% (v/v) *Lactobacillus plantarum*. At 0 day the pH and titratable acidity (T.A) were 6.98±0.00 and 0.38±0.01% respectively and were ranged from 7.21±0.01 to 4.41±0.01 and 0.22±0.02 to 1.19±0.06 respectively. After 5days of storage, significant ($p<0.01$) decrease in pH and corresponding increase in T.A in both treated and untreated samples were observed. However, the decrease in pH and increase T.A were less pronounced in refrigerated (mean=10^{o}C) probably due to the low temperature that hindered the proliferation of lactic acid bacteria responsible for lactic acid production. The untreated unrefrigerated sample (A) had the lowest (4.41±0.01) pH and highest (1.19±0.06%) T.A whereas treatment F had the highest (6.89±0.01) pH and lowest (0.23±0.01%) T.A at the end of the storage(5days).The increase in pH and decreased in T.A observed in C was attributed to alkaline nature of *kanwa* which act as buffering agent couple with its antimicrobial activity, while the increase in pH and decrease T.A observed in E despite the fact that it was not treated with any additives was due to the refrigeration temperature that slow down the metabolic activities of LAB. This results agreed with the findings of Sokolinska *et al* ,(2004) who reported that the pH values of samples milk decreased during the manufacturing process, from the time it was inoculated with bacterial cultures to the time when it was manufactured ranging from 6.7 to 4.34. Murevanhema (2012) reported a fairly stable pH for fermented bambara milk beverage (probiotic yoghurt) during storage period at 5 °C, and a significant decrease in pH during storage at 15 and 25°C. Gradual increase in T.A of bambara nut milk beverage during storage at the 5 °C and rapid increase in T.A was reported when the samples were stored at 15 and 25°C (Murevanhema 2012). The decreased in pH and simultaneous increased T.A of the pasteurised milk during the storage period could be attributed to the starter culture's activity, such as post acidification due to formation of lactic acid or growth of the bacteria used during fermentation (Osundahunsi *et al.*, 2007).

The results of viscosity showed that, at 0day, all the samples had same viscosity (10.00 cp) and mean values ranged from 57±5.77cp to 10±0.00cp. After 1day of storage, significant ($p<0.05$) increase was observed in all the samples with the exception of refrigerated samples (E, F, and G) which increased slightly. At day one of storage, the viscosity of A, B, C, D, were 57±5.77, 30±2.00, 28±2.00 and 18±2.00 CP respectively, while the refrigerated samples (at 10°C) had the same viscosity (14±0.00cp).Marked increased in viscosity especially in sample A (untreated) may be attributed to the action of starter culture (*L. fermentum and L. plantarum)* role in the production of exocellular texturing agents called exopolysaccharides that might interact with the protein content of milk and increase the viscosity and rheological quality of products. The increase is less pronounced in samples stored at refrigerated temperature (10.00cp to 14.00cp). This finding are in lined with the report of Abu-Jdayil and Mohameed (2002) who reported an increase in the apparent viscosity of concentrated yogurt during storage. At 2 days of storage decreased in viscosity was observed in both samples throughout the storage period except those under refrigerated storage that remained stable. The stability of viscosity observed in refrigerated samples may be related to low temperature that negatively affected the metabolic activities of *L plantarum and L. fermentum* responsible for fermentation of pasteurised milk as evident from their pH and T.A.

The mean of lactic acid bacteria (LAB) count of pasteurised milk inoculated with 2% *L. plantarum* were presented in Table 3. At 0 hour, the mean LAB count was 5.08 log cfu/ml. Significant ($p<0.05$) count reduction were observed in all samples stored at both storage condition with the exception of untreated sample(A) that showed increasing trend throughout the storage period. With regard to the unrefrigerated samples, sample D showed highest count reduction (0.34 log cfu/ml) while B showed lowest count reduction (0.09 log cfu/ml) at 3days of storage. For refrigerated (mean temperature=10°C) samples, highest count reduction (0.82 log cfu/ml) was recorded in sample G while lowest count reduction was observed in E (0.30 log cfu/ml) at 4days of storage. At the end of storage

period (5days), significant ($p<0.05$) increase in LAB count were observed in all samples stored at ambient temperature with the exception of D that showed decreasing trend. Highest increase (0.39 log cfu/ml) was recorded in sample (A) while lowest increased was observed B (0.10 log cfu/ml).Count reduction observed in treatments B and C might be attributed to antibacterial activity of potash (*kanwa*) while count reduction recorded in sample D may be due to antimicrobial action of sodium benzoate. Lowest count reduction observed in refrigerated sample could be attributed to the low temperature that hinder the proliferation and metabolic activities of *L. fermentum* and *L. plantarum* which grow well at temperature between 20 and 40 °C with an optimum temperature range of 30–32°C (Sengupta *et al.*, 2013) .Antimicrobial activity of *kanwa* and sodium benzoate also played important role in hindering the growth of LAB in refrigerated samples. Similar findings were made by previous researchers such as Aminigo *et al* (2009) who reported decrease in the lactic acid bacteria count in African yam bean yoghurt stored at refrigeration temperature for 4 weeks. Furthermore, Muhammed *et al.*, (2009) reported that soy yoghurt stored at 37°C contained higher LAB count than those at refrigerated temperature after 7 days of storage.

Table 1: Change in physicochemical properties of treated and untreated pasteurised milk inoculated with 2%(v/v) *L. fermentum L. plantarum* at different storage intervals

Treatments								
		Unrefrigerated				Refrigerated		
Parameters	Time(Day)	A	B	C	D	E	F	G
pH	0	6.98±0.00	6.98±0.00	6.98±0.00	6.98±0.00	6.98±0.00	6.98±0.00	6.98±0.00
	1	6.35 ± 0.01^{a}	6.98 ± 0.01^{bcd}	7.00 ± 0.02^{bc}	6.96 ± 0.02^{bd}	7.02 ± 0.02^{bc}	7.21 ± 0.01^{ad}	6.97 ± 0.01^{bcd}
	2	4.68 ± 0.01^{a}	5.19 ± 0.01^{b}	6.10 ± 0.01^{c}	6.32 ± 0.03^{d}	6.98 ± 0.01^{e}	7.14 ± 0.01^{f}	6.93 ± 0.01^{g}

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	3	4.63±0.02[a]	4.68±0.00[b]	5.32±0.02[c]	4.95±0.01[d]	6.88±0.01[e]	7.07±0.01[f]	6.87±0.01[g]
	4	4.49±0.02[a]	4.57±0.01[a]	4.97±0.01[b]	4.88±0.02[b]	6.87±0.02[c]	7.03±0.01[a]	6.87±0.01[c]
	5	4.41±0.01[a]	4.51±0.01[a]	4.81±0.01[a]	4.68±0.01[a]	6.85±0.01[b]	6.89±0.01[b]	6.85±0.01[b]
Titratable acidity	0	0.38±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38±0.01
	1	0.58±0.01[a]	0.40±0.01[b]	0.37±0.02[c]	0.41±0.02[d]	0.36±0.02[c]	0.22±0.02[d]	0.24±0.00[d]
	2	0.65±0.03[a]	0.49±0.01[b]	0.45±0.02[c]	0.47±0.02[b]	0.37±0.02[d]	0.22±0.02[e]	0.23±0.00[e]
	3	0.71±0.01[a]	0.63±0.02[b]	0.61±0.01[c]	0.60±0.01[c]	0.39±0.01[d]	0.23±0.00[e]	0.23±0.00[e]
	4	0.90±0.02[a]	0.70±0.02[b]	0.64±0.01[c]	0.65±0.03[c]	0.40±0.03[d]	0.23±0.00[e]	0.23±0.00[e]
	5	1.19±0.06[a]	0.85±0.02[b]	0.71±0.02[c]	0.79±0.02[d]	0.41±0.02[e]	0.23±0.01[f]	0.24±0.0[f]
Viscosity (Cp)	0	10±.0.00	10±.0.00	10±.0.00	10±.0.00	10±.0.00	10±.0.00	10±.1.15
	1	57±5.77[a]	30±2.00[b]	28±2.00[c]	18±2.00[d]	14±0.00[f]	14±0.00[f]	14±0.00[f]
	2	50±2.00[a]	26±0.00[b]	14±0.00[cd]	12±1.15[cde]	12±2.00[de]	12±2.00[de]	12±1.52[de]
	3	20±0.00[a]	12±0.00	12±0.00	12±0.00	12±0.00	12±0.00	12±0.00
	4	14±0.00[a]	10±0.00[b]	10±0.00[b]	10±0.00[b]	12±0.00[b]	12±0.00[c]	12±0.00[c]
	5	10±0.00[a]	10±0.00[a]	10±0.00[a]	10±0.00[a]	12±0.00[b]	12±0.00[b]	12±0.00[b]

A= Unpreserved (negative control) B =0.2 % Potash (v/v) , C= 0.4% Potash (v/v) D= 0.2% Sodium benzoate, E= untreated , F= 0.2% Potash (v/v), G = 0.2% Sodium benzoate. Means with different superscripts in the same row are significantly different ($P<0.05$)

The mean of overall acceptability pasteurised milk inoculated with 2% *L. plantarum* treated with additives were indicated in Table 4. The results

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indicated that at 0 hour the untreated sample had 9.0±0.1 acceptability with 100 % likeness. Significant ($p<0.05$) decrease in

Table 3: The mean of Lactic Acid Bacterial count (log cfu/ml) of treated and untreated pasteurised milk inoculated with 2%(v/v) and *L. fermentum L. plantarum* at different storage intervals

Treatments							
	Unrefrigerated				Refrigerated		
Time(Day)	A	B	C	D	E	F	G
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.12	0.08*	0.09*	0.23*	0.48*	0.57*	0.76*
2	0.22	0.16*	0.19*	0.30*	0.36*	0.52*	0.80*
3	0.25	0.09*	0.11*	0.34*	0.36*	0.49*	0.82*
4	0.30	0.01*	0.05*	0.29*	0.30*	0.49*	0.82*
5	0.39	0.10	0.08	0.28*	0.30*	0.36*	0.78*

Key: A= Untreated (negative control) B= 0.2g Bean seed (BS), C= 0.4g BS, D= 0.3g Red pepper (RP),E=0.6g RP, F= 0.2% Potash (v/v), G =0.4% Potash (v/v) ,and H= 0.2% Sodium benzoate (positive control). The values are means of triplicate determination.). Means followed by * indicated log count reduction. Significant different at $P<0.05$.

Overall acceptability in all samples at the end of the storage (5 days) were observed with the exception of G that remained virtually stable. All the samples stored at ambient condition(35^{o}C) received 100% likeness by the panelist at 1- 2days of storage with the exception of untreated sample(A) that had 80% likeness whereas refrigerated samples (E, F and G) showed 100% preference by the judges from 1-days of storage. Highest score (8.7±0.9) was recorded in G while lowest scores (5.3±1.7) was recorded in A at the end of storage time (5 days).The refrigerated samples were rated best with 100% preference compared to the samples stored under ambient temperature.This could be attributed to low temperature which is below the optimum level of *Lb plantarum*(20-40 ^{o}C) that affected its fermentative capability as evident in their neutral pH throughout the storage period. Decrease in likeness(40%) to sample A probably due to

its lower pH higher T.A and poor texture while decreased in likeness(40%) to C could be attributed to high concentration of *kanwa*(0.4%) that affected its colour.

Table 4: The mean sensory scores (Overall acceptability) of treated and untreated pasteurised milk inoculated with 2% *L. plantarum and L. fermentum* at different storage interval.

Treatments							
	Unrefrigerated				Refrigerated		
Time(Day)	A	B	C	D	E	F	G
0	9.0±0.1(100)	9.0±0.1(100)	9.0±0.1(100)	9.0±0.1(100)	9.0±0.1(100)	9.0±0.1(100)	9.0±0.1(100)
1	7.4±0.3(100)	7.7±0.4(100)	7.7±0.4(100)	8.2±0.4(100)	8.9±0.4(100)	8.9±0.4(100)	9.0±0.1(100)
2	7.3±0.3(80)	7.4±0.5(100)	7.0±0.6(80)	7.5±1.4(100)	8.2±0.4(100)	8.3±0.4(100)	9.0±0.5(100)
3	5.9±2.0(80)	6.7±1.1(80)	6.5±1.1(80)	7.0±0.7(100)	8.0±0.5(100)	8.3±0.5(100)	9.0±0.5(100)
4	5.5±0.8(60)	6.2±0.6(80)	6.1±0.5(60)	6.7±0.5(100)	7.9±0.5(100)	7.9±0.5(100)	8.9±0.3(100)
`5	5.3±1.7(40)	5.6±1.7(60)	5.4±1.5(40)	6.2±0.9(80)	7.7±1.1(100)	7.5±1.3(100)	8.7±0.9(100)

Key: A= Unpreserved (negative control) B= =0.2 % Potash (v/v) ,C= 0.4% Potash (v/v) D= 0.2% sodium benzoate. E= untreated, F= 0.2% Potash (v/v), G = 0.2% Sodium benzoate. Significant different at $P<0.05$. Values are means ±SD of five determinations. The figures enclosed in bracket represents percentage of likeness

Hedonic Scale ; 9= like extremely, 8=like very much, 7= like moderately, 6 = like slightly, 5= neither like nor dislike, 4= dislike slightly, 3= dislike moderately, 2= dislike very much, and 1= dislike extremely

Conclusion and recommendation:

Base on the results obtained, refrigeration in combination with preservatives (potash) were effective means of preserving pasteurised milk. All the concentration of potash (*kanwa)* used were shown to exhibit preservation potential of milk though, 0.2% was the best under both storage condition. *L. fermentum and L. plantarum* could also be used as potential starter culture. This practice adopted by some rural dwellers/milk hawkers in area where refrigerator facilities are not available is recommended. Too much concentration of *kanwa* could affect

the organoleptic quality of milk. (≥0.4%). Further study should be carryout with *kanwa* in preserving other dairy products especially besides pasteurized milk.

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